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Validating the positivity thresholds of drug-tolerant anti-infliximab and anti-adalimumab antibody assays

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Summary

Background: When used proactively, drug-tolerant anti-tumour necrosis factor (TNF) antibody assays provide early opportunity to suppress immunogenicity.

Aim: To validate positivity thresholds of IDKmonitor drug-tolerant anti-infliximab and -adalimumab antibody assays.

Methods: We applied positivity thresholds, defined by testing sera from 498 anti-TNF naive healthy adults, from the Exeter Ten Thousand study to data from our therapeutic drug monitoring (TDM) service and Personalised Anti-TNF Therapy in Crohn's disease (PANTS) cohort to explore associations with drug level and treatment outcomes.

Results: The 80% one-sided lower confidence interval of the 99th centile concentration for anti-infliximab and –adalimumab antibodies were lower than the manufacturers threshold of 10 arbitrary units (AU)/mL; 9 and 6 AU/mL, respectively. Using these new thresholds in the TDM cohort, more adalimumab- than infliximab- (11.2% [814/7272] vs 3.1% [390/12 683] $P < 0.0001$) treated patients were reclassified as antibody-positive. Adalimumab drug concentrations in this reclassified group (median 8.1, interquartile range [IQR] 5.5-11.0 mg/L) were lower than those below the new threshold (≤5AU/mL) (median 9.9, IQR 7.1-13.0 mg/L; $P < 0.0001$), but higher than at the manufacturer's threshold (10-29 AU/mL) (median 5.9 mg/L, IQR 3.5-8.7; $P < 0.0001$). No difference in infliximab drug concentration was observed using the new or manufacturer's positivity threshold ($P = 0.11$). In the PANTS cohort, patients with anti-adalimumab antibody concentrations at or above the new threshold were more likely to be in primary non-response (25/68 [37%] vs. 64/332 [19%], $P = 0.0035$), and non-remission at week 54 (51/62 [82%] vs. 168/279 [60%], $P = 0.0011$), than patients with anti-drug antibody concentrations in the group below the new threshold (0-5 AU/mL); this was not seen for anti-infliximab antibodies.

Conclusion: Laboratories should derive antibody positivity thresholds for assays they use. For adalimumab, low-concentration anti-drug antibodies were associated with lower drug levels and treatment failure.

Rachel Nice and Neil Chanchlani contributed equally to this work as co-first authors and RN and NC joint first and MP and NAK joint last.

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1 | INTRODUCTION

Biopharmaceuticals, or biologics, are large complex proteins manufactured in, or derived from, living sources. The anti-tumour necrosis factor (TNF) therapies, infliximab and adalimumab, are the most widely used biologics for treating immune-mediated diseases, including inflammatory bowel disease, and in 2018, they accounted for an expenditure in excess of $29 billion in the United States alone.[1] Repeated administration, however, often induces the formation of anti-drug antibodies that lead to drug clearance and treatment failure.[2-5]

Pharmacokinetic therapeutic drug monitoring (TDM) in patients with inflammatory bowel disease, improves durability of response, safety and cost-effectiveness of anti-TNF therapy, compared to empirical dosing alone.[6-9] Debate remains, however, how best to measure drug and anti-drug antibody levels and whether TDM is best undertaken proactively during routine follow-up, or whether reactive TDM at the time of loss of response is adequate.[10] Recent data support proactive TDM because it allows optimisation of drug levels and earlier detection of anti-drug antibodies, which provides a window of opportunity for clinicians to suppress immunogenicity by introducing an immunomodulator.[9,11-16]

Enzyme-linked immunosorbent assays (ELISAs) are the most commonly used analytical methods for the measurement of anti-TNF drug and anti-drug antibody levels.[17,18] Most studies have reported results using 'drug-sensitive' or 'free' antibody assays. 'Drug-tolerant' or 'total' antibody assays include a pre-analytical acid antibody-drug disassociation step. This allows antibodies to be detected earlier, at a potentially reversible stage, when drug is still present. These assays are therefore ideally suited for proactive TDM.[17]

The Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust uses the Immundiagnostik AG (IDKmonitor) drug-tolerant anti-infliximab and anti-adalimumab antibody assays for its national TDM service. The positivity threshold is defined by the manufacturer as 10 arbitrary units (AU)/mL. We sought to validate this positivity threshold for both assays and to describe the relationship between drug and anti-drug antibody levels and clinical outcomes using these new positivity thresholds.

2 | METHODS

2.1 | Study design

We designed three related studies in mutually exclusive cohorts:

- To validate the positivity thresholds of the IDK drug-tolerant anti-TNF antibody assays, we tested sera from healthy individuals who had not been exposed to anti-TNF therapies (EXTEND cohort).
- To explore the relationship between drug and anti-drug antibody levels and the impact on clinical reporting at the new positivity threshold we used paired drug and antibody data from our TDM Clinical Service (Exeter TDM cohort).
- To test whether anti-drug antibody concentrations using the new positivity thresholds were associated with treatment failure, we reanalysed data from the prospective Personalised Anti-TNF Therapy in Crohn's disease study at the new positivity threshold (PANTS cohort).

2.2 | Participants and outcome definitions

2.2.1 | Validating the positivity threshold

The Exeter Ten Thousand (EXTEND) cohort is a prospective cohort study with a recallable biorepository designed to understand genetic contributions to common diseases. To be included, adult volunteers needed to live within 25 miles of the city of Exeter in the South West of England, United Kingdom (EXTEND; www.exeter10000.org). Participants were invited to a single 30-minute appointment when they completed a short self-reported questionnaire about their health and lifestyle and provided urine and blood samples. We tested sera from a random sample of 498 healthy volunteers from this cohort for antibodies to infliximab and adalimumab, who were not taking regular medications and had never been exposed to anti-TNF therapies. We validated the positivity thresholds as the 80% one-sided lower confidence interval of the 99th centile of antibody concentration in the EXTEND cohort, as per the United States Food and Drug Administration and European Medicines Agency guidelines for validating confirmatory assays.[19,20]

2.2.2 | Exploring the relationship between drug and antibody levels

The Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust provides an anti-TNF TDM clinical service to hospitals throughout the UK. Requests come from physicians who work in a variety of disciplines; the majority are from gastroenterologists administering anti-TNF therapy for inflammatory bowel disease. Clinicians are asked to send trough drug levels, but no clinical data are linked to TDM test requests. We applied the new positivity thresholds to anti-infliximab and –adalimumab antibody results from the Exeter laboratory TDM cohort.[21] We compared drug levels in antibody-positive patients using the manufacturer's threshold and the new threshold.

In all patients with paired drug and anti-drug antibody results at the time of last testing, we assigned the proportion of patients who had *clearing* (antibody positive, drug negative; <0.8 mg/L) and *non-clearing anti-drug antibodies* (antibody positive, drug positive; ≥0.8 mg/L) using the new thresholds compared to using the manufacturers threshold.

In order to explore the effect of lowering the diagnostic positivity threshold on the prevalence of transient antibodies, in patients

who had multiple anti-drug antibody tests, we classified the proportion of patients who had consistently *negative* (all antibody tests negative); *transient* (a single positive test with subsequent negative test); *single last-test positive* (last test positive with no subsequent antibody measurements) and *persistent* (at least two positive tests) anti-drug antibodies.

2.2.3 | Investigating antibody positivity and treatment failure

PANTS is a UK-wide, multicentre, prospective observational cohort reporting the treatment failure rates of the anti-TNF drugs infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar CT-P13 [Celltrion]), and adalimumab (Humira [Abbvie]) in 1610 anti-TNF naive patients with active luminal Crohn's disease.[3] Treatment failure endpoints were primary non-response at week 14 and non-remission at week 54. Primary non-response was defined as exit for resectional surgery or corticosteroid use at week 14. Patients who exhibited both a failure of C-reactive protein to fall to ≤3 mg/L or by 50% from baseline and a failure of Harvey Bradshaw Index[22] to fall to ≤4 or by 3 points were also classified as primary non-response. For children, a failure of short Pediatric Crohn's Disease Activity Index[23] to fall to <15 or by more than 12.5 points was used. Response and grey zone were intermediate categories based on improvements in symptoms and/or C-reactive protein, respectively Remission was defined at week 14 and 54 as a C-reactive protein of ≤3 mg/L and Harvey Bradshaw Index of ≤4 points (short Pediatric Crohn's Disease Activity Index ≤15), without ongoing steroid therapy or exit for treatment failure.

2.3 | Laboratory methods

All laboratory analyses were performed at the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust. Anti-TNF drug and anti-drug antibodies were measured on a Dynex Technologies (Chantilly, Virginia, USA) DS2 automated ELISA platform.

2.4 | Drug-tolerant anti-TNF antibody assays

The Immundiagnostik (IDK) AG (Bensheim, Germany) IDKmonitor infliximab (K9654) and adalimumab (K9651) total anti-drug antibody assays allow semi-quantitative measurement of both free and bound anti-drug antibodies.[24,25] A pre-treatment acid dissociation step is used to separate anti-drug antibodies from the therapeutic antibody. The assay then follows a standard ELISA format using recombinant therapeutic antibody as a capture and detection antibody. For both assays, the manufacturer established a positivity threshold by linear dilution of sera with high concentrations of anti-TNF antibody until no further linear dilution was possible; 10 AU/ml for both assays. The manufacturer then validated the anti-TNF antibody threshold in sera from 40 anti-TNF naive individuals.

The infliximab and adalimumab total anti-drug antibody assays have measuring ranges of 4.5-400 AU/mL and 5.5-200 AU/mL, respectively. Based on analysis of pooled patient serum quality control, the intra-assay coefficient of variation is ≤8.7% at 11.8 AU/mL for the infliximab total anti-drug antibody assay (n = 128), ≤13.16% at 12.7 AU/mL for adalimumab antibodies (n = 130). The manufacturer's recommended positivity threshold for both total anti-TNF drug antibody assays is 10 AU/mL.

2.5 | Anti-TNF drug level assays

The IDKmonitor free infliximab (K9655) and adalimumab (K9657) drug level assays permit quantitative measurement of free therapeutic drug in serum. The assays follow a standard ELISA format using a specific monoclonal anti-drug antibody fragment as a capture antibody and peroxidase-labelled anti-human IgG antibody as a detection antibody. The measuring range for both assays is 0.8-45 mg/L, with absence of drug being defined using a cut-off of <0.8 mg/L.

2.6 | Statistical analysis

Statistical analyses were undertaken in R version 3.6.3 (R Foundation for Statistical Computing). All analyses were two tailed, unless otherwise stated, and $P < 0.05$ were considered significant. Summary descriptive statistics are presented as median and interquartile ranges for continuous variables and as percentages for categorical variables.

2.7 | Validating the positivity threshold

We constructed cumulative distribution plots of anti-drug antibody concentrations from the EXTEND cohort and used bootstrapping to calculate the 80% one-sided lower confidence interval of the 99th centile to define anti-infliximab and anti-adalimumab antibody assay threshold.[19,20]

2.8 | Exploring the relationship between drug and antibody levels

To visualise the relative effects of changing from the manufacturer's positivity thresholds to the newly validated thresholds, we also constructed cumulative distribution plots of anti-infliximab or—adalimumab antibody concentrations in all patients at the time of last testing in the Exeter TDM cohort. We used pairwise Mann-Whitney U tests to compare median drug concentrations in patients with increasing anti-drug antibody concentrations.

Anti-drug antibody levels for each drug were categorised as follows: positive using the new positivity threshold, positive using the manufacturer's threshold, and based on cut-offs established in the PANTS study; moderate and high antibody concentrations (30-99 AU/mL and ≥100 AU/mL respectively).[3] Differences between proportions of patients with clearing, non-clearing, transient and persistent anti-drug antibodies using the manufacturers and the newly validated positivity thresholds, were sought using chi-squared analyses.

2.9 | Investigating antibody positivity and treatment failure

We collapsed the predefined treatment outcomes from the PANTS study—grey zone and response, into the remission category at week 14. We used chi-squared analyses to detect differences in rates of primary non-response at week 14 and non-remission at week 54 between patients with increasing antibody concentrations using the categories described above.[3]

2.10 | Ethical considerations

In line with Health Research Authority guidelines, formal ethics approval for our TDM service evaluation was not required.[26] The sponsor of both the EXTEND and PANTS studies is the Royal Devon and Exeter NHS Foundation Trust. The South West Research Ethics Committee approved both studies (REC Reference: 14/SW/1089 for Exeter 10,000; November 2009, REC Reference: 12/SW/0323 for the PANTS study; January 2013). Patients were involved in the design of both the EXTEND and PANTS cohorts.

3 | RESULTS

3.1 | Defining the positivity threshold

We obtained sera from 498 healthy volunteers who had not been exposed to anti-TNF therapies: 54.0% (269/498) were female, 91.4% (455/498) were white European, with a median age of 48 (interquartile range [IQR] 39-58) years. Overall, 5.2% (26/498) were current smokers. At inclusion 39.4% (196/498) individuals were overweight (Body Mass Index 25-29.9 kg/m^2) and 14.5% (72/498) were obese (Body Mass Index >30 kg/m^2).

Cumulative distribution plots for anti-TNF drug concentrations in the healthy volunteers from the EXTEND cohort are shown in Figure 1A,B. The 80% one-sided lower confidence interval of the 99th centile concentrations for anti-drug antibodies to infliximab and adalimumab were 9 and 6 AU/mL, respectively, both lower than the manufacturers recommended threshold of 10 AU/mL (the point estimate of the 99% centiles were 10 AU/mL for antibodies to infliximab and 6 AU/mL for antibodies to adalimumab).

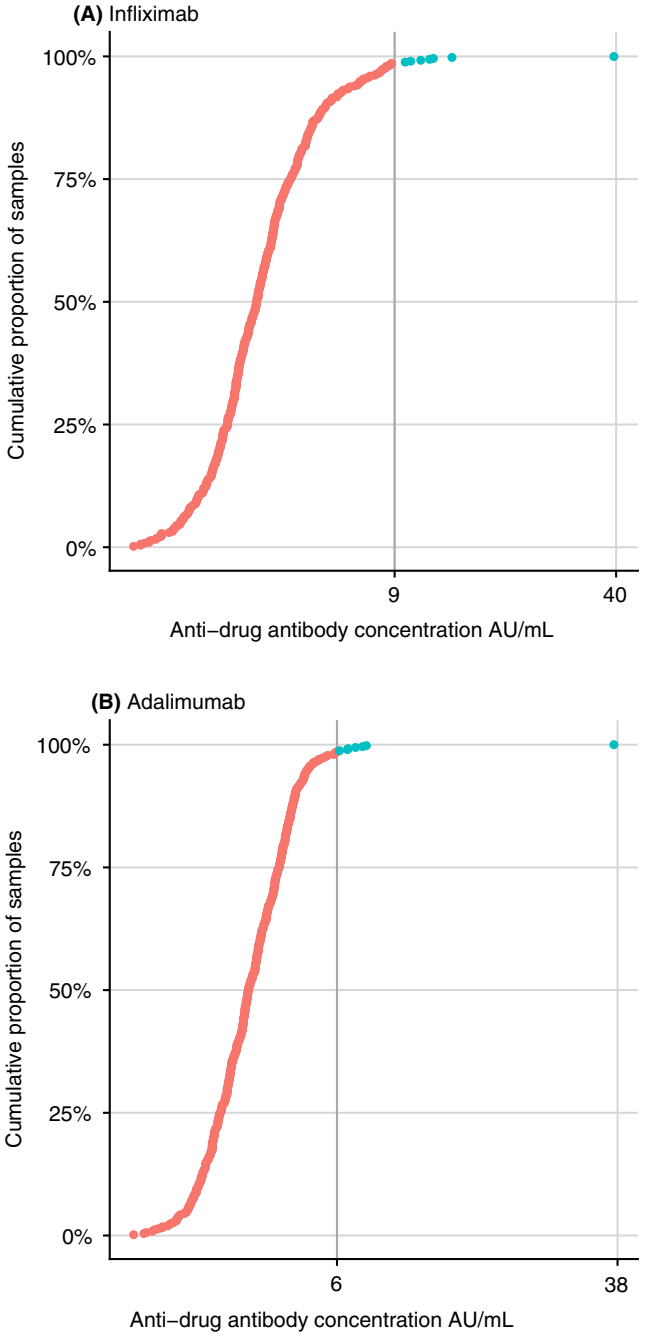

FIGURE 1 (A and B) Cumulative distribution plots of anti-drug antibody concentrations (on a log scale) in 498 biologic-naive healthy volunteers using our drug-tolerant anti-infliximab (A) and anti-adalimumab (B) assays respectively. The vertical line denotes the 80% one-sided lower CI of the 99th centile

3.2 | Exploring the relationship between drug and antibody levels

Between January 2012 and December 2019, 32 490 paired infliximab and 11 830 adalimumab drug and anti-drug antibody assays in 12 683 and 7272 patients, respectively were analysed as part of the routine TDM service in Exeter.

At the time of last testing, immunogenicity was more common in infliximab- than adalimumab-treated patients, irrespective of whether we used the manufacturers or the newly validated positivity threshold (Figure 2A,B). Using the manufacturer's threshold of 10 AU/mL, anti-infliximab antibodies were detected in 47.8% (6068/12 683) patients compared to 24.4% (1771/7272) adalimumab-treated patients ($P < 0.0001$). The proportion of patients reclassified as positive with anti-drug antibodies using the newly validated positivity thresholds (infliximab 9 AU/mL and adalimumab 6 AU/mL), was greater in adalimumab (11.1% [814/7272]) than infliximab (3.1% [390/12 683])-treated patients ($P < 0.0001$). Reducing the positivity threshold resulted in more patients classified with non-clearing antibodies to both infliximab (manufacturer's threshold 26.7% (3390/12 683) vs. newly validated threshold 29.4% (3733/12 683) $P < 0.0001$) and adalimumab (manufacturer's threshold 15.8% (1146/7272) vs. newly validated threshold 26.7% (1941/7272) $P < 0.0001$): but had no effect on the proportions of patients with clearing antibodies, to either drug (Table 1).

In total, 6170 and 2673 patients had more than one anti-infliximab and anti-adalimumab antibody level tested, respectively. The median number of tests per patient was 3 (range: 2-4) for infliximab- and 2 (range: 2-3) for adalimumab-treated patients.

Reducing the positivity threshold resulted in more patients being classified with persistent anti-drug antibodies to both infliximab (manufacturer's threshold 41.3% [2551/6170]) vs. newly validated threshold 44.8% [2765/6170] $P < 0.0001$) and adalimumab (manufacturer's threshold 16.5% (440/2673) vs. newly validated threshold 26.3% (704/2673) $P < 0.0001$). The proportions of adalimumab-, but not infliximab-treated, patients whose last and only anti-drug antibody test was positive or who had transient antibodies increased following the reclassification of anti-drug antibody test results (Table 1).

The effect of progressively increasing anti-drug antibodies on infliximab and adalimumab drug concentrations is shown in Figure 3A,B, respectively. Adalimumab concentrations in the newly reclassified positive group (6-9 AU/mL) were lower (median adalimumab concentration 8.1, IQR 5.5-11.0 mg/L), than in the group below the new threshold (≤5 AU/mL) (median adalimumab concentration 9.9, IQR 7.1-13.0 mg/L; $P < 0.0001$) but were not as low as in the group above the manufacturer's threshold (10-29 AU/mL) (median adalimumab concentration 5.9, IQR 3.5-8.7 mg/L; $P < 0.0001$). There was no significant difference between infliximab concentrations for patients with an anti-infliximab concentration of 9 AU/mL (the group reclassified with the lowered threshold) and those with an anti-infliximab concentration of < 9 AU/mL ($P = 0.11$).

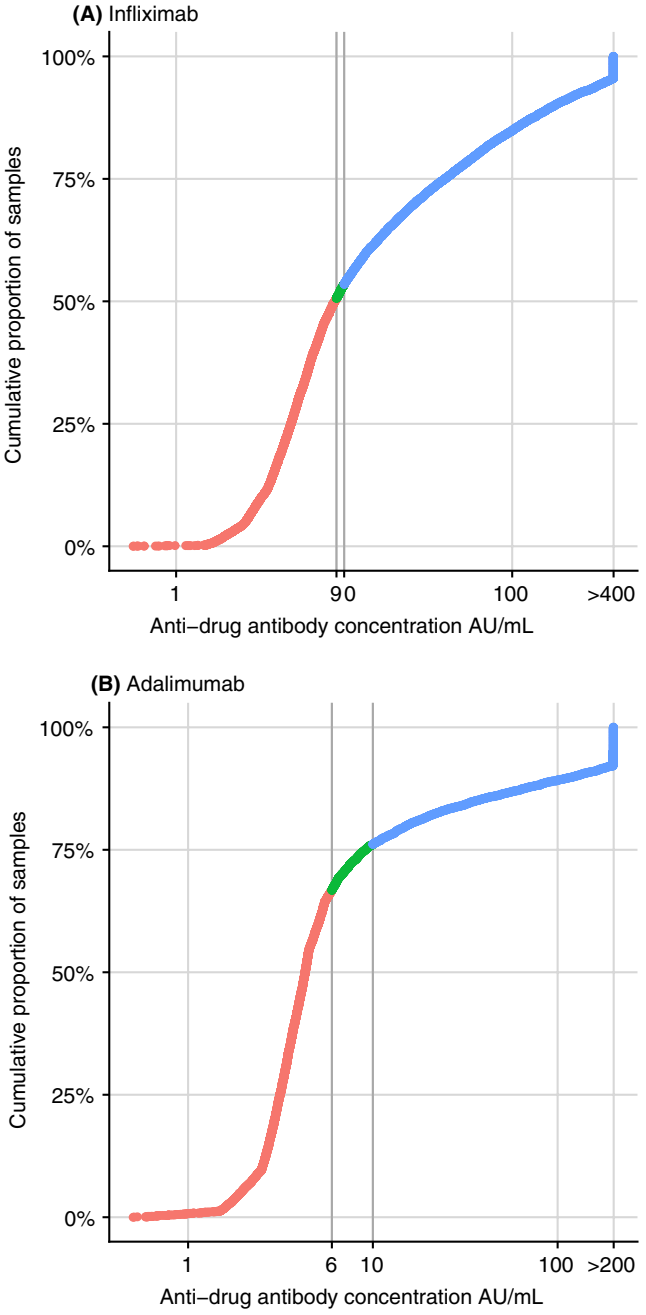

FIGURE 2 (A and B) Cumulative distribution plots of anti-drug antibody concentrations (log scale) measured by the Exeter therapeutic drug monitoring service from January 2012 to December 2019 from infliximab- (A) n = 12 683 and adalimumab- (B) n = 7272 treated patients. Vertical lines indicate our newly validated positivity thresholds of 9 and 6 AU/mL for infliximab and adalimumab, respectively, and the manufacturer's threshold of 10 AU/mL. Samples in pink are those less than the newly validated threshold in green are those between the newly validated and manufacturer's threshold and in blue are those above the manufacturer's threshold

3.3 | Investigating antibody positivity and treatment failure

The difference between the new anti-infliximab antibody positivity threshold (9 AU/mL) and manufacturer's threshold (10 AU/mL) is very small. When applied to the PANTS cohort only 1.7% (11/658) infliximab-treated patients would be reclassified as antibody positive at week 14 compared to 5.7% (24/420) for adalimumab-treated patients. In view of the small proportion of infliximab-treated

TABLE 1 Antibody status, stratified by type of drug and applied threshold, in patients tested in the Exeter therapeutic drug monitoring cohort

	Infliximab			Adalimumab		
Antibody status	Manufacturer's threshold (10 AU/mL)	Newly validated threshold (9 AU/mL)	P^a	Manufacturer's threshold 10 AU/mL)	Newly validated threshold (6 AU/mL)	P^a
Patients tested						
Clearing[b]	21.1% (2678/12 683)	21.5% (2725/12 683)	0.48	8.6% (625/7272)	8.9% (644/7272)	0.60
Non-clearing[c]	26.7% (3390/12 683)	29.4% (3733/12 683)	<0.0001	15.8% (1146/7272)	26.7% (1941/7272)	<0.0001
Patients with more than one sample						
Negative[d]	40.8% (2515/6170)	36.9% (2278/6170)	<0.0001	70% (1872/2673)	53.6% (1434/2673)	<0.0001
Transient[e]	8.8% (540/6170)	8.6% (530/6170)	0.77	6.8% (182/2673)	9.5% (255/2673)	0.0003
Single last test positive[f]	9.1% (564/6170)	9.7% (597/6170)	0.97	6.7% (179/2673)	10.5% (280/2673)	<0.0001
Persistent[g]	41.3% (2551/6170)	44.8% (2765/6170)	<0.0001	16.5% (440/2673)	26.3% (704/2673)	<0.0001

[a]Chi-square test performed.
[b]Positive anti-drug antibody result with an undetectable drug level.
[c]Positive anti-drug antibody result with a detectable drug level.
[d]All anti-drug antibody tests negative.
[e]A single positive anti-drug antibody test with subsequent negative test.
[f]Last anti-drug antibody test positive with no subsequent anti-drug antibody measurements.
[g]At least two anti-drug antibody-positive tests.

patients reclassified as positive in the PANTS cohort, the relationship between antibody positivity and treatment failure has not been investigated in this group. Adalimumab-treated patients on combination therapy with an immunomodulator were less likely to develop anti-drug antibodies above our new threshold of 6 AU/mL compared to patients on monotherapy with adalimumab only ($P < 0.0001$; Table 2).

Week 14 adalimumab drug concentrations in the reclassified positive group (6-9 AU/mL), were lower (median 7.6, IQR 6.1-9.1 mg/L) than in the group below the new threshold (0-5 AU/mL) (median 11.5, IQR 8.7-14.8 mg/L, $P < 0.0001$), but were not as low as individuals above the manufacturer's threshold (10-29 AU/mL) (median 5.8, IQR 2.1-8.0 mg/L, $P = 0.035$; Figure 4).

Using the prespecified outcome definitions from PANTS, at week 14, patients with anti-adalimumab antibody concentrations at or above the new threshold were more likely to be in primary non-response (25/68 [37%] vs. 64/332 [19%], $P = 0.0035$), and non-remission at week 54 (51/62 [82%] vs. 168/279 [60%], $P = 0.0011$), (Figure 5A,B) than patients with anti-drug antibody concentrations in the group below the new threshold (0-5 AU/mL).

4 | DISCUSSION

4.1 | Key findings

We have demonstrated that the positivity thresholds for the IDKmonitor drug-tolerant anti-infliximab and anti-adalimumab antibody assays are lower than the manufacturer's suggested threshold of 10 AU/mL for both infliximab (9 AU/mL) and adalimumab (6 AU/mL). This was done in a cohort of almost 500 anti-TNF naive individuals from the Exeter 10 000 study.

Immunogenicity was more common in infliximab than adalimumab-treated patients. The new anti-drug antibody thresholds, however, differentially increased rates of persistent, non-clearing anti-drug antibodies for adalimumab-treated patients. Anti-TNF anti-drug antibody concentrations above the newly validated, but below the manufacturer's recommended positivity thresholds were associated with intermediate drug concentrations for adalimumab. In the PANTS cohort this translated to higher rates of primary non-response and non-remission at week 54 in adalimumab-, but not infliximab-treated patients.

4.2 | Interpretation

Because antibody responses are heterogeneous, there is a lack of standardised antibody testing material meaning that manufacturers define positivity thresholds in small cohorts of healthy individuals.[17,18] There are several potential explanations to account for why the new positivity thresholds for both anti-TNF antibody assays were lower than the manufacturer's recommended thresholds. Most importantly, our sample was more than 10 times larger than the manufacturer's original cohorts,[24,25] meaning that we were able to report positivity thresholds with greater precision: in particular, for the anti-adalimumab assay where the prevalence, and variance of anti-drug antibody concentrations, were less than the anti-infliximab concentrations. Furthermore, compared to the manufacturer's original convenience cohorts, our selection of patients without any comorbidities from the Exeter 10 000 cohort were less likely to

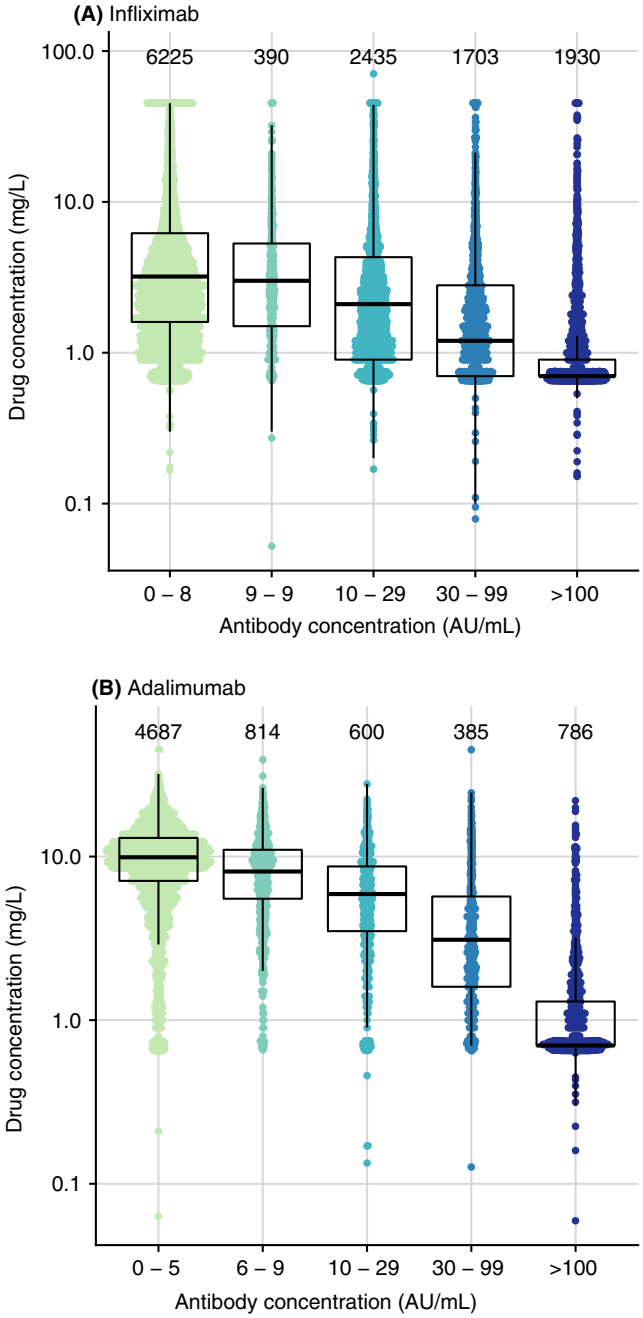

FIGURE 3 (A and B) Bee-swarm box and whiskers plot showing anti-infliximab (A) and anti-adalimumab (B) antibody concentration plotted against drug concentration for samples received through the Exeter therapeutic drug monitoring service

TABLE 2 Anti-adalimumab antibody concentration stratified at week 14 by immunomodulator use at baseline

Antibody concentration (AU/mL)	Immunomodulator (n = 227)	No immunomodulator (n = 193)
<6	205/227 (90%)	140/193 (73%)
6-9	6/227 (3%)	18/193 (9%)
10-29	9/227 (4%)	15/193 (8%)
30-99	3/227 (1%)	7/193 (4%)
>99	4/227 (2%)	13/193 (7%)

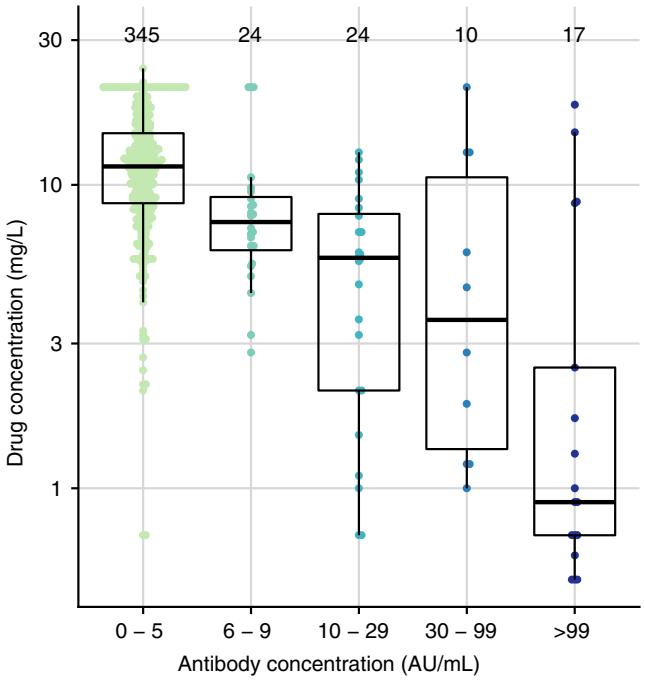

FIGURE 4 Bee-swarm box and whisker plot showing anti-drug antibody concentration against adalimumab concentration for 420 samples received in the first year of the PANTS study

have had cross-reactive anti-allotype antibodies such as rheumatoid factor.[27,28]

The reasons why we see a larger difference between the manufacturer's and the new positivity thresholds for the anti-adalimumab than anti-infliximab antibody assays are less clear. One explanation may relate to differences in the prevalence of pre-formed antibodies to the drugs.[29,30] Because of recognition of xenotopes in the mouse variable domains of the chimeric antibody, as a result of environmental exposure to rodents, pre-formed antibodies are more commonly detected by anti-infliximab than anti-adalimumab antibody assays.[31,32]

Establishing the prevalence and clinical impact of transient anti-drug antibodies across studies is limited by a lack of standardised nomenclature and differences in type and drug-tolerance of the assays used.[33-35] In this study, we have shown that lowering the positivity thresholds of the IDKmonitor anti-TNF antibody assays would not lead to a clinically meaningful increase in reporting of transient anti-drug antibodies. The significance of reporting a higher prevalence of persistent, non-clearing antibodies when lowering the positivity threshold is less clear. We recognise that there will always be a balance between test sensitivity and specificity; using the manufacturer's positivity thresholds, these were not well defined or validated. We benchmarked specificity on 99% based on international guidelines, however, there is a potential for the newly classified group to be false positives. Equally, increasing test sensitivity by

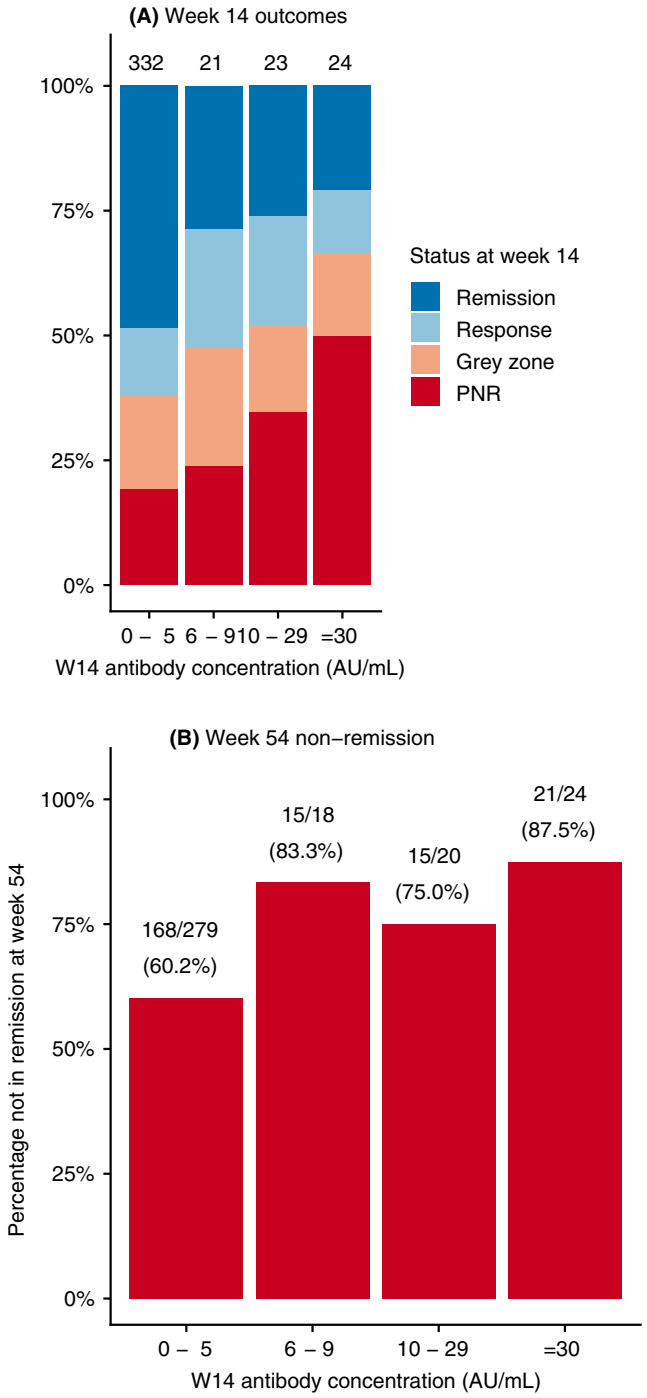

FIGURE 5 (A) Stacked barchart showing proportion of adalimumab-treated patients in the PANTS study meeting criteria for predefined treatment outcomes stratified by week 14 anti-adalimumab antibody concentration and (B) barchart showing the proportion of adalimumab-treated patients in the PANTS study in remission at week 54 stratified by week 14 antibody concentration

reducing the positivity threshold may allow detection of true positives earlier in their development before leading to drug clearance.

In the PANTS study,[3] like in other studies,[34,36-38] immunogenicity only impacted clinical outcome if the antibodies led to drug clearance. Studying the function of non-clearing antibodies is hampered by analytical difficulties of excluding drug from ex-vivo samples whilst maintaining a functional antibody product.[17,18,39] Further work is needed to understand their natural history; for example, do non-clearing antibodies eventually clear drug with further maturation; do they neutralize drug; or are they simply bystanders? For now, earlier detection of anti-drug antibodies may allow the introduction of an immunomodulator, or anti-TNF dose optimisation, to mitigate immunogenicity. Because these antibodies may be false positives or transient, repeat testing should occur before treatment changes.

4.3 | Limitations

The Exeter TDM cohort is a non-selected clinical referral cohort and although we recommend that blood sampling occurs just before the next dose, inevitably, some non-trough samples will have been processed. Because anti-drug antibody assays are not completely drug tolerant, this is likely to bias the data by underestimation of rates of immunogenicity.[40] This effect may be more important in adalimumab-treated patients where TDM testing is more often ad-hoc rather than immediately before administration of drug. In addition, we have only studied the IDKmonitor assays here: users of other assays should consider validating their positivity thresholds using similar methodologies. Finally, in the PANTS cohort, we used pragmatic definitions of remission closely aligned to routine treatment targets: we accept that our data would have been strengthened by endoscopic outcomes.

4.4 | Generalisability

As over 90% of participants in both the Exeter 10 000 and PANTS studies were white European, it is highly likely that our findings using the IDKmonitor anti-TNF drug-tolerant antibody assays are generalisable to other cohorts of white European patients with inflammatory bowel disease. Whether our results are generalisable to other ethnicities, where rates of anti-drug antibody formation are lower, is less certain.[41,42] Furthermore, whether lower thresholds are clinically relevant in other immune-mediated disorders, such as rheumatoid arthritis, where autoantibodies frequently cross-react in anti-drug antibody ELISA assays, is also unknown.[31] Manufacturers of other assays should consider validating their positivity thresholds using similar methodologies.

5 | CONCLUSIONS

Laboratories should independently derive antibody positivity thresholds for assays they use as demonstrated here for the IDKmonitor drug-tolerant anti-drug antibody assays. Our findings suggest that lowering the positivity threshold of the anti-adalimumab antibody assay to 6 AU/mL may add value to the use of this test. Changing to the lower thresholds differentially increased the rates of persistent, non-clearing antibodies to both infliximab and adalimumab.

Anti-drug antibody concentrations above the newly validated thresholds, but below the manufacturer's threshold, were associated with intermediate drug concentrations that were related to treatment failure in adalimumab- but not infliximab-treated patients.

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DATA AVAILABILITY

The data underlying this article are available in the article.

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